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PRELIMINARY SCREENING FOR POTENTIAL ALGICIDES

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The search for new and more efficient bactericides, fungicides, insecticides, and herbicides has become increasingly extensive and intensive. Special laboratories have been established for the continuous screening of chemicals for evidence of their toxicity to the various living organisms. The quest for algicides, however, has lagged far behind. This is due, in part, to the lack of any routine procedures for culturing algae which could be utilized in a screening process. Any investigation on mixed growths of algae is difficult to evaluate and virtually impossible to duplicate. While it is recognized that experiments with unialgal cultures cannot replace final tests in the field, it is essential that such cultures be employed for rapid screening tests and for investigations where variable factors must be held to a minimum.

The demand for both general and selective algicides is becoming more definite as technicians and officials connected with water treatment plants increase their interest in controlling algae responsible for various nuisance problems. In more and more situations it is becoming desirable to control or destroy particular kinds of algae while, at the same time, permitting other useful or non-troublesome types to remain unaffected. It is not economically sound to waste an algicide on many species of algae or to destroy the whole biota when destruction of only one species is desired.

Copper sulfate and chlorine have long been the only algicides used extensively in water supplies (American Water Works Association, 1950). With algicides, even more so than with bactericides and fungicides, cheapness is a very important factor, since the algicide has to be used in large quantities to treat the entire water supply. It is difficult, therefore, to discover new materials which can compete with copper sulfate and chlorine in this respect.

NEW ALGICIDES

The increasing interest in new algicides has caused a number of research centers in educational and industrial institutions to undertake a search for new chemicals that may be selected for widespread use (Bowser, 1951; Williams *et al.*, 1952). Announcement of the effectiveness of 2,3-dichloronaphthoquinone in the control of bloom-forming blue-green algae is a good example (Fitzgerald *et al.*, 1952). Journals concerned primarily with the care of swimming pools, cooling tanks, farm fishponds, and other bodies of water are now beginning to carry announcements and advertisements of special algicides with various materials as the active agents.

The present need is to develop satisfactory techniques for the discovery and evaluation of chemicals with algicidal properties. In addition, a mass of experimental data is required before the screening tests can be steered into the most promising channels. With this in mind, the staff at the Robert A. Taft Sanitary

Engineering Center has experimented with laboratory cultures of algae in developing a procedure for determining the toxicity of various types of chemicals to algae.

The testing procedure involves a preliminary screening of each chemical against a few algal cultures which is then followed by a more extensive laboratory and field study of the most effective chemicals. In addition, before new algicides can be recommended for use, it is necessary to give consideration to a number of other important factors such as the toxicity of the potential algicides to fish and mammals, their cost, availability, ease of handling, and stability.

The results reported in this paper are limited to the preliminary screening tests, where each chemical is used at only one concentration and against only six cultures of algae. A report on the more extensive tests will be available later.

PROCEDURE FOR SCREENING

For the preliminary screening, the chemicals were tested at a concentration of 2 p.p.m. of the gross materials (except where otherwise indicated). For the most part, the chemicals selected for testing were those with a history of toxicity to plants or animals and especially to microorganisms. A number of the compounds tested are available commercially as fungicides, bactericides, herbicides, and insecticides.

The six representative cultures of algae which were selected for the preliminary screening tests included *Cylindrospermum licheniforme* B. and F. and *Microcystis aeruginosa* Ktz. representing the blue-green algae (Myxophyceae), *Scenedesmus obliquus* (Turp.) Ktz. and *Chlorella variegata* Beijerinck representing the green algae (Chlorophyceae), and *Gomphonema parvulum* (Ktz.) V. H. and *Nitzschia palea* (Ktz.) W. Smith representing the diatoms (Bacillarieae). In a few cases *Gloeocapsa dimidiata* (Ktz.) Dr. and D. was substituted for the closely related *Microcystis aeruginosa*. All cultures used were unialgal and were selected for their ability to produce rapid and uniform growth under laboratory conditions.

The culture medium approximated Gerloff's modification of Chu No. 10 with the amount of nitrate doubled (Palmer and Maloney, 1953). Tests were carried out in 25 ml. Erlenmeyer flasks and incubated at a constant temperature of 22° C in an illuminated culture room (Palmer, 1952). Sterile, double strength medium was inoculated with a small amount (one-fifteenth to one-thirtieth by volume) of an actively growing culture of the alga. A 7.5 ml. portion of this inoculated medium was then combined with a like quantity of distilled water containing 4 p.p.m. by weight, in the case of solids, or 0.0004 percent by volume in the case of liquids, of the chemical to be tested. This resulted in a final chemical concentration of 2 p.p.m. (or 0.0002%) in normal strength medium and with an inoculum of one-thirtieth to one-sixtieth the volume of the original algal culture. The number of algal cells in the test medium at the beginning of the test was comparatively small, averaging approximately 125,000 per ml.

During the period of incubation, the amount of visible algal growth was recorded at specified intervals for a total of 21 days. Growth was then compared with that in the control flasks containing 15 ml. of normal strength inoculated culture medium but no test chemical. The results for each chemical were then recorded under the following headings:

- Toxic (T) Where no growth occurred in the presence of the test chemical, but did occur in the control flask.
- Partially Toxic (P) Where growth occurred in the presence of the test chemical, but the amount was not as great as that in the control flask.
- Non-Toxic (N) Where growth in the presence of the test chemical was similar in amount to that in the control flask.
- Stimulant (S) Where growth in the presence of the test chemical was greater in amount than that in the control flask.

RESULTS

The results of the research are summarized in table 1 and show, for each test chemical, the relative toxicity (T, P, N, S) evident after incubation periods of 3, 7, 14, and 21 days for each of the six cultures of algae. The table shows results obtained with a total of 76 test chemicals, including such well known compounds as copper sulfate, calcium hypochlorite, mercuric acetate, 2,4 dichlorophenoxyacetic acid (2,4-D), 2,3 dichloronaphthoquinone, tetrachlorophene, benzene hexachloride, 3-(p-chlorophenyl) -1, 1-dimethylurea (CMU), rosin amine D acetate (RADA), and streptomycin. The 76 compounds are arranged in ten groups, based upon their chemical composition and structure. Chemicals preceded by an asterisk were obtained as commercially advertised toxic products (insecticides, herbicides, algicides, bactericides, and fungicides).

TABLE 1
Toxicity of Chemicals to Algae

	Cylindrospermum				Microcystis				Scenedesmus				Chlorella				Gomphonema				Nitzschia			
PERIOD OF INCUBATION (IN DAYS)	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21
INORGANIC SALTS																								
*Copper sulfate (anhydrous).....	P	P	T	T	T	T	T	T	P	P	N	N	T	T	T	T	T	T	T	T	T	T	T	T
*Copper sulfate (with stabilizing agent).....	T	T	T	T	T	T	T	T	P	P	P	N	P	P	P	P	N	T	T	T	T	N	T	T
Colloidal silver (33% silver nitrate).....	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
*Activated colloidal silver compound.....	P	P	N	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
*Calcium hypochlorite.....	T	P	N	N	T	T	T	T	T	P	N	N	T	T	T	T	T	T	T	T	T	T	T	T
Sodium chloride.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ORGANIC SALTS																								
*Disodium copper salt of ethylene diamine-tetra acetic acid (18.25%).....	N	T	N	N	N	T	N	P	N	N	N	N	N	T	N	T	N	N	N	N	N	T	N	T
*Copper salt plus citrate.....	N	T	T	T	T	T	T	T	P	P	N	N	N	T	N	T	N	N	N	N	N	T	N	T
Copper naphthenate (8%).....	N	P	N	N	T	T	T	T	P	N	N	N	P	N	N	N	T	T	P	P	N	N	N	N
*Zinc dimethyl dithiocarbamate (65%).....	P	P	N	N	T	T	P	N	N	N	N	N	P	P	P	N	T	T	T	N	T	T	T	T
Zinc dimethyl dithiocarbamate (100%).....	T	T	T	T	T	T	T	T	T	T	T	P	T	T	T	T	T	T	T	T	T	T	T	T
*Disodium ethylene bisdithiocarbamate (19%).....	N	S	N	N	N	T	N	P	N	N	N	N	T	N	P	N	T	T	T	N	T	T	T	T
*Pentachlorophenate (75%) plus sodium salts of other phenols.....	P	P	P	P	P	P	P	N	P	P	P	N	N	N	N	N	P	P	N	P	T	P	N	P
*Sodium pentachlorophenate (75%), sodium salts of other phenols (13%).....	T	T	P	N	T	T	P	N	P	P	N	N	N	N	N	N	P	P	N	N	T	T	N	P
*p-chlorophenyl-p-chlorobenzenesulfamate.....	P	N	N	N	P	P	P	N	N	P	N	N	N	P	P	N	N	P	N	N	T	P	N	N
Xanthic acid, ethyl sodium salt.....	N	N	N	N	N	P	P	N	N	N	N	N	N	P	P	N	N	N	N	N	N	N	N	N
*Lauryl isoquinolinium bromide (20%).....	T	P	N	N	T	T	T	T	T	T	T	T	T	P	P	N	N	P	N	N	N	P	N	N
Mercuric acetate.....	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
Phenyl mercuric hydroxide.....	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
Phenyl mercuric nitrate.....	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
ORGANIC ACIDS																								
Acetic acid.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4, chloro-o-toloxoacetic acid.....	T	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N
2, 4 dichlorophenoxyacetic acid.....	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N
Iodoacetic acid.....	P	P	N	N	T	T	T	T	T	T	P	N	N	N	N	N	N	P	P	P	N	N	N	N
*2, 4, 5 trichlorophenoxyacetic acid.....	S	S	N	N	T	T	N	N	N	N	N	N	N	S	S	N	N	N	N	P	P	N	N	N
3 nitro 4 acetoxybenzoic acid.....	S	S	S	N	S	S	S	N	N	N	N	N	N	S	S	S	N	N	N	P	P	S	S	N
3 nitro 4 hydroxybenzoic acid.....	S	S	S	N	S	S	S	N	N	N	N	N	N	S	S	S	N	N	N	P	P	S	S	N
3 nitro 4 methoxybenzoic acid.....	N	N	N	N	P	P	N	N	N	P	P	N	N	P	N	N	N	P	P	P	S	S	N	N

TABLE 1—(Continued)

	Cylindrospermum				Microcystis				Schedesmus				Chlorella				Gomphonema				Nitzschia			
PERIOD OF INCUBATION (IN DAYS)	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21
ALCOHOLS, ALDEHYDES, KETONES																								
4, 4 dichloro-alpha-methylbenz- hydrol.....	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P	N	N	N	N	N
*Terpine alcohol (85% pine oil).....	T	N	N	N	P	P	P	N	N	N	N	N	T	N	N	N	T	N	N	N	N	N	N	N
*di (p-chlorophenyl) methyl carbinol.....	P	P	N	N	N	N	N	N	T	T	T	T	T	P	N	N	T	T	P	T	T	T	T	T
2, 3 dichloronaphthoquinone.....	P	N	N	N	P	N	N	N	T	T	T	T	T	P	N	N	T	T	P	P	P	P	P	P
Salicylaldehyde.....	P	N	N	N	P	N	N	N	P	N	N	N	P	P	N	N	T	T	P	P	P	P	P	P
Vanillin.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Dimethylaminobenzaldehyde.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PHENOLS																								
Tetrachlorophene.....	T	P	N	N	N	N	N	N	P	P	N	N	P	N	N	N	N	N	N	N	T	N	N	N
2 tertiary-butyl-4, 6 dinitro- phenol.....	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	S	N	N	N	N	N	N	P
Dinitro-secundary butylphenol.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Picric acid.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SUBSTITUTED HYDROCARBONS																								
Benzene hexachloride, alpha isomer.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Benzene hexachloride, beta isomer.....	P	P	N	N	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Benzene hexachloride, delta isomer.....	N	N	N	N	N	P	N	N	P	P	N	N	N	N	N	N	P	T	T	T	P	P	P	N
Benzene hexachloride, gamma isomer.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
*Benzene hexachloride, gamma isomer, tech.....	N	N	N	N	N	N	P	P	N	N	P	N	N	N	N	N	P	P	P	P	N	N	N	N
Cumene hydroperoxide (commercial).....	P	P	N	N	T	T	N	N	N	N	N	N	T	P	N	N	N	P	N	N	T	T	N	N
*Dichloro diphenyl trichloroe- thane.....	N	N	N	N	T	T	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N
2-benzoyl 1, 3 dichloropropane (50% active).....	T	T	P	P	T	T	T	T	P	P	N	N	T	T	T	N	T	T	P	T	T	T	T	N
*Chlorinated camphene (60%).....	P	P	P	P	T	T	T	T	P	P	P	N	T	T	T	N	T	P	T	P	T	P	T	N
*Chlorinated benzene (No. 1).....	T	T	T	P	T	T	T	T	T	T	P	P	T	T	T	N	T	T	P	T	T	T	T	N
*Chlorinated benzene (No. 2).....	T	T	P	P	T	T	T	T	T	N	P	N	P	N	N	N	T	T	P	T	P	T	T	N
*Chlorinated benzene (No. 3).....	T	T	T	T	T	T	T	T	T	N	P	N	N	P	N	N	T	T	T	T	T	T	T	T
*Alkyl aryl bromide, aqueous solution.....	N	N	N	N	T	T	T	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
QUARTERNARY AMMONIUM COMPOUNDS																								
*Methyldodecylbenzyl tri- methyl ammonium chloride (50%) 50% H ₂ O.....	T	P	N	N	P	N	N	N	T	T	T	N	P	P	N	N	T	T	T	N	T	T	T	N
*Cetyltrimethyl ammonium bro- mide (2%), alkylate ether alcohol (10%), 85% inert.....	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
*Dodecylacetamido dimethyl benzyl ammonium chloride (100%).....	P	P	P	N	T	P	P	N	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
*High molecular alkyl-dimethyl- ammonium chlorides (10%).....	P	P	N	N	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N	N	P	N	N	N
*Mixed trimethyl and trimethy- loctadecadienyl ammonium chlorides (25%), 75% H ₂ O.....	N	N	N	N	N	N	N	N	N	P	N	N	N	S	N	N	N	N	N	N	N	N	N	N
*Methyl dodecyl benzyl tri- methyl ammonium chloride plus tridecyl methyl hydroxy ethyl imidazolium chloride (10%), 90% H ₂ O.....	N	N	N	N	N	N	N	N	P	P	P	N	P	P	N	N	N	N	N	N	N	N	N	N
AMINES, AMIDE DERIVATIVES																								
Alpha naphthylamine.....	N	P	N	N	T	T	T	T	P	P	N	N	T	P	N	N	T	T	P	P	T	P	N	N
Beta naphthaquinoline.....	P	P	P	P	N	N	N	N	P	P	P	P	P	P	N	N	T	T	P	P	P	P	P	P
2, 4 dinitrophenylhydrazine.....	N	N	N	N	P	P	P	N	P	P	P	N	N	P	N	N	P	P	N	N	P	P	N	N
Thiocarbamide.....	N	N	N	N	P	P	P	N	P	P	P	N	N	P	N	N	P	P	N	N	P	P	N	N
*3-(p-chlorophenol)-1, 1-di- methylurea.....	P	P	T	T	T	T	T	T	T	T	P	P	T	P	P	N	P	P	P	T	T	T	T	T

TABLE 1—(Continued)

	Cylindrocapsa				Microcystis				Scenedesmus				Chlorella				Gomphonema				Nitzschia			
PERIOD OF INCUBATION (IN DAYS)	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21	3	7	14	21
ROSIN AMINE COMPOUNDS																								
*Rosin amine D acetate (50% solution).....	T	T	T	T	T	T	T	T	P	P	N	N	T	T	T	T	T	T	T	T	T	T	T	T
*Rosin amine D sulphate, wettable powder (75% active).....	T	T	T	T	T	T	T	T	T	T	T	P	T	T	T	T	T	T	T	T	T	T	T	T
Diethanol rosin amine D acetate (70% active).....	T	T	T	T	T	T	T	T	N	P	P	P	T	T	P	N	T	T	T	T	T	T	T	T
Emulsifiable rosin amine D (85% active).....	T	T	T	T	T	T	T	T	T	P	P	N	T	T	T	T	T	T	T	T	T	T	T	T
Emulsifiable rosin amine D pentachlorophenate (40% active).....	T	T	P	N	P	P	P	N	N	N	N	N	T	T	N	N	T	T	P	N	T	T	T	T
Sodium carboxyethyl rosin amine (10% solution).....	N	N	P	N	P	P	P	N	N	N	N	N	N	N	N	N	T	N	N	N	N	N	N	N
Emulsified rosin amine derivative (40% active).....	P	P	N	P	T	T	P	N	P	P	P	P	P	N	N	N	T	T	T	T	T	T	T	T
N (3-aminopropyl) rosin amine D diacetate (28% active).....	T	T	T	P	T	T	T	T	P	P	P	P	T	T	T	P	T	T	T	T	T	T	T	T
ANTIBIOTICS																								
*Acti-dione.....	P	P	N	N	N	N	N	N	T	T	T	T	T	P	N	N	N	T	T	T	T	T	T	T
*Aerosporin-Polymyxin B (sulfate).....	P	P	P	T	T	T	T	T	T	T	T	N	T	T	T	T	T	T	T	T	T	T	T	T
*Penicillin G, potassium (crystalline).....	P	P	N	N	T	T	T	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
*Streptomycin sulfate.....	T	T	T	T	T	T	T	T	P	P	T	T	N	N	N	N	N	N	N	N	N	T	T	T
*Terramycin.....	P	P	N	N	T	T	T	T	N	N	N	N	N	N	N	N	N	N	N	N	T	P	N	N

*Obtained as commercial product

T Toxic

P Partially toxic

N Non-toxic

S Stimulant

N¹, P¹, T¹ *Gloeocapsa* substituted for *Microcystis*

Comparative Toxicity of Chemical Groups

Four of the ten groups of chemicals are outstanding in containing several chemicals which were toxic to the algae. These groups are the Inorganic salts, Organic salts, Rosin amine compounds, and Antibiotics. Many of the other groups contain individual compounds which are also toxic to the algae at a concentration of 2 p.p.m.

Copper sulfate and calcium hypochlorite, which are representatives of the first group, proved to be toxic, or at least partially toxic to all six of the test algae. Both were less effective against *Scenedesmus* than they were against the other genera of algae. This is in agreement with the statement by Bussy (1949) that *Scenedesmus obliquus* is highly resistant to copper sulfate and chlorine.

Another representative of group one, colloidal silver (33% silver nitrate), was highly toxic to all six of the algae. However, in a commercial preparation of an activated colloidal silver compound, there was relatively no toxic effect. This could be due, in part, to the difference in the concentrations of the active ingredients of the two compounds. Sodium chloride had no inhibitory effect on any of the six algae.

Among the Organic salts, the mercuric compounds were highly toxic to the algae. However, they are relatively high in cost and their probable toxicity to animals may limit their possible usefulness as algicides. The copper salt and citrate mixture was toxic to all of the test algae except *Scenedesmus*. This formulation would not appear, however, from these preliminary tests to be of any advantage over copper sulfate when used alone. A 2 p.p.m. concentration of zinc dimethyl dithiocarbamate was toxic to the six test algae while a commercial

preparation, which is recommended as a fungicide, containing 65 per cent of this compound was toxic to only three of the algae. The two pentachlorophenate compounds had some effect on every alga except *Chlorella*, but no marked effect in any case. Lauryl isoquinolinium bromide differed from the others in that it was selectively toxic to *Scenedesmus*.

As stated above the Organic acids, as a group, had little effect on the six algae. Additional study might be warranted in the case of iodoacetic acid as a possible algicide for *Microcystis* and its related forms. It appeared to have relatively the same toxicity as 2,3 dichloronaphthoquinone which has already been described (Fitzgerald *et al.*, 1952) as a promising algicide.

Of the ten groups, the Phenols were the least toxic to the algae. One compound, tetrachlorophene, was capable of temporarily preventing or reducing growth of four of the six algae. However, within one or two weeks this initial toxicity had been overcome.

The compounds in the group listed as Alcohols, Aldehydes, and Ketones were sporadic in their toxic effects and none of the compounds appeared to be outstanding in their algicidal properties.

Four isomers of benzene hexachloride are included in the group listed as Substituted hydrocarbons. The delta isomer was the only one of these which exhibited much toxicity. It was toxic to *Gomphonema* and partially toxic to *Nitzschia*. The compound 2-benzoyl-1,3 dichloropropane (50%) displayed some toxicity to all six algae. This was only a partial effect in the case of *Scenedesmus* and its growth returned to normal after two weeks. Chlorinated camphene (60%) had some toxic effect on all the algae except *Chlorella*. However, the only pronounced effect was against *Microcystis*. The three chlorinated benzene compounds were in fair agreement in their toxicity to the six algae. One, however, did prove to be toxic to *Chlorella* while the others did not. The aqueous solution of alkyl aryl bromide was selectively toxic to *Microcystis*.

One of the Quaternary ammonium compounds, dodecylacetamido dimethyl benzyl ammonium chloride, showed greater toxicity to the green algae than to the blue-green algae. This is of particular interest because, in general, the green algae were found to be resistant to most of the tested chemicals.

The dimethylurea compound listed in the eighth group, Amines and Amide derivatives, was toxic to all six algae. In two cases the effect became more intensified after prolonged incubation.

The Rosin amine compounds were highly toxic to the test algae at 2 p.p.m. of the gross concentration. The least effective formulation may have been so as a result of its low percentage of active ingredients. Lawrence (1954) reports dehydroabietylamine acetate (Rosin amine D acetate), at 0.5 p.p.m. of the active ingredient, to be toxic to the filamentous green alga, *Pithophora*, and non-toxic to phytoplankton, but the particular genera of the organisms comprising the phytoplankton are not given.

Among the Antibiotics, polymyxin was clearly the most toxic to the algae. Algal growth was prevented in most cases, for the total incubation period of 21 days. Results with streptomycin confirm previous studies (Foter *et al.*, 1953) which indicate that it is particularly effective against blue-green algae.

Comparison of Effects on Each Alga

For a minimum of three of the four observation periods during incubation, a total of 26-29 of the chemical compounds prevented growth of *Microcystis*, *Nitzschia*, and *Gomphonema*. For the remaining three algae this same condition was limited to 12-16 chemicals. It is evident, therefore, that the blue-green alga, *Microcystis*, and the two diatoms, *Gomphonema* and *Nitzschia*, were much more sensitive to the various chemicals than were the other blue-green alga, *Cylindrospermum*, and the two green algae, *Scenedesmus* and *Chlorella*.

In general, the two diatoms reacted somewhat alike to the test chemicals. The results with the Organic salts, zinc dimethyl dithiocarbamate (65%) and disodium ethylene bisdithiocarbamate (19%) as well as with the substituted hydrocarbon, benzene hexachloride, delta isomer, would indicate that they may be selective for diatoms. Streptomycin sulfate was the only substance which was decidedly toxic to *Nitzschia* and non-toxic to the other diatom, *Gomphonema*.

The two green algae tested were frequently alike in their reactions to the chemicals. Exceptions to this are evident in the cases of copper sulfate, copper salt plus citrate, calcium hypochlorite, 2-benzoyl, 1,3 dichloropropane (50%), chlorinated benzene (No. 1), and some of the Rosin amine compounds where *Scenedesmus* was more resistant than *Chlorella*. *Chlorella*, on the other hand, was more resistant than *Scenedesmus* to lauryl isoquinolinium bromide (20%), di(p-chlorophenyl) methyl carbinol, methyl dodecylbenzyl trimethyl ammonium chloride (50%), and acti-dione.

The two blue-green algae which were used, in contrast to the green algae and diatoms, were extremely different from one another in their reactions to the test chemicals. *Microcystis* proved to be much more sensitive than *Cylindrospermum*. The following substances were selectively toxic to *Microcystis*: copper naphthenate (8%), iodoacetic acid, alpha naphthylamine, chlorinated camphene (60%), penicillin and terramycin. Tetrachlorophene appeared to be temporarily toxic to *Cylindrospermum* and non-toxic to *Microcystis*.

Additional Observations and Comments

A distinct initial stimulation in the growth of most of the algae was noted in the flasks containing a concentration of 2 p.p.m. of the Organic acids, 3 nitro 4 acetoxybenzoic acid and 3 nitro 4 hydroxybenzoic acid. A closely related compound, 3 nitro 4 methoxybenzoic acid, produced no stimulation and was partially toxic to 4 of the 6 algae.

The length of the period of incubation is an important consideration in determining the effects of the chemicals on algal cultures. For all six of the test algae the number of substances toxic to them in the short observation periods was greater than that for the longer periods. With *Chlorella*, for example, 25 chemicals were toxic to the alga for 3 days, but only 14 were effective in preventing growth up to 21 days. However, this difference was much less for the blue-green algae.

Up to the present time there are few data available to determine whether or not insecticides, herbicides, bactericides, and fungicides may also be effective algicides. The dimethylurea formulation which is listed as a universal herbicide, was toxic to the algae at the concentration employed. Two other herbicides, 2,4,5 trichlorophenoxyacetic acid and 2,4 dichlorophenoxyacetic acid had little or no effect on the six algae. Most of the insecticides tested had little effect on the algae, the chlorinated camphene probably being the most toxic of the group.

Bactericides and particularly fungicides may be more toxic to algae than are many of the pesticides. Some of these, such as acti-dione, 2,3 dichloronaphthoquinone, and the quaternary ammonium compound, dodecylacetamido dimethyl benzyl ammonium chloride, were selective in their action and may form types from which selective algicides could be developed.

It is important to emphasize the fact that the results reported here are based upon a certain procedure involving the use of 2 p.p.m. of the gross concentrations of chemical substances being tested, the addition of the chemical at the time of inoculation with the algae, the use of six specific cultures of algae, and the employment of a single culture medium having a relatively high pH. The modification of any of these or other factors could produce results differing greatly from those which are reported here.

In later studies it is planned to modify certain of the more important factors

and to determine which ones may be of particular significance in effecting the screening tests.

SUMMARY

A comparatively simple procedure for the preliminary screening of chemical compounds for algicides is described together with results obtained with the first 76 substances tested.

As groups, inorganic and organic salts, rosin amine compounds, and antibiotics tended to show high toxicity to the six test algae at gross concentrations of 2 p.p.m.

A number of the tested substances exhibited selective toxicity to one group of algae or to one particular alga.

In general, the algae most sensitive to the test chemicals were the blue-green alga, *Microcystis*, and the two diatoms, *Nitzschia* and *Gomphonema*. The algae most resistant to the test chemicals were the green algae, *Scenedesmus* and *Chlorella*, and the blue-green alga, *Cylindrospermum*.

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